C1 types of metastases, particularly epithelial malignancies. Thus, techniques designed to alter EphA2 expression can be exploited to diagnose and treat metastatic disease.

Please replace the paragraph beginning at page 6, line 4, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Hybridomas producing antibodies specific to EphA2 have been selected. Use of the RIMMS technique has resulted in the production of a multiplicity of hybridomas producing monoclonal antibodies that specifically bind EphA2. To date, at least 450 hybridomas have been identified which produce antibodies capable of distinguishing malignant from normal cancer cells. Of the first four such hybridomas to be characterized, two recognize independent epitopes C2 on EphA2. The first, D7, produces an antibody recognizing an intracellular epitope. The second, B2D6, produces an antibody that specifically binds an extracellular epitope of EphA2, a characteristic that enables its effective use for the diagnosis and treatment of selected metastatic tumors. Murine hybridomas B2D6 and D7 were deposited on December 8, 2000, with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and assigned ATCC Numbers PTA-2754 and PTA-2755, respectively.

Please replace the paragraph beginning at page 6, line 18, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

It is known in the art to use antibodies to detect the presence or overexpression of a specific protein. Because EphA2 is overexpressed in metastatic cells, EphA2-specific antibodies C3 of this invention may be used to detect this overexpression and, thus, to detect metastatic disease. Such techniques include but are not limited to western blotting, precipitation, agglutination, and ELISA assays. These techniques are well known in the art. Also, the

extracellular epitope specificity of EphA2-specific antibodies of this invention can be exploited to detect changes in EphA2 localization which are associated with metastasis. In normal breast and prostate epithelial cells, EphA2 is enriched in within cites of cell adhesion, whereas in metastatic cells, EphA2 distribution is altered. In metastatic prostate cells EphA2 is diffusely distributed, and in metastatic breast cancer cells EphA2 is redistributed into the membrane ruffles. EphA2 expression is also known to be altered in lung and colon malignancies, and it is believed that EphA2 altered expression occurs in other types of metastasis, particularly epithelial malignancies Techniques such as immunohistological staining or immunofluorescent microscopy are well known in the art and may be used to visualize EphA2 distribution. See, for example, U.S. Patent No. 5,514,554, hereby incorporated by reference. In order to detect overexpression or altered distribution of EphA2, the EphA2-specific antibodies may be labeled covalently or non-covalently with any of a number of known detectable labels, such as fluorescent or radioactive substances, as is known in the art. Alternatively, a secondary antibody specific for the antibodies of this invention is labeled with a known detectable label and used to detect the EphA2-specific antibodies in the above techniques. Thus, the antibodies of this invention provide methods to detect metastatic transformation.

Please replace the paragraph beginning at page 8, line 30, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

Following the RIMMS strategy using tyrosine phosphorylated proteins from Ras-transformed human epithelial cells, hybridomas were screened, and an antibody specific for EphA2 has been isolated. This antibody, B2D6, was used to assess the levels of EphA2 expression in nontransformed prostatic epithelial cells and prostatic tumor cells. Low levels of EphA2 expression were found in non-transformed prostatic epithelial cells, but this EphA2 expression was enriched within sites of cell-cell contact and interacted with cell-bound ligand. Compared to non-transformed cells, two features distinguish EphA2 in metastatic prostate

cancer cells: 1) EphA2 is overexpressed; 2) EphA2 is diffusely distributed and does not appear to interact with ligand. To confirm these data, western blots were performed using the EphA2 specific antibodies. EphA2 overexpression in human prostate cancer cells (LNCAP, DU145, PC3) directly correlates with their invasiveness *in vitro* and *in vivo*. Of the three lines tested, LNCAP is the least aggressive, DU145 is more aggressive, and PC3 is the most aggressive. As seen in Fig. 2, DU145 cells exhibit higher levels of EphA2 expression than LNCAP, and PC3 cells exhibit even higher levels of EphA2 expression. Similarly, as shown in Figs. 2B and 2C, EphA2 expression is elevated in variants of human prostatic epithelial cells transformed by oncogenic K-Ras or X-irradiation. The three lanes in Fig. 2B show "normal" MCL prostatic epithelial cells, and K-Ras and X-ray transformed cell lines derived therefrom. Similarly, the three lanes of Fig. 2C show "normal" 267B1 prostatic epithelial cells, and K-Ras and X-ray transformed cell lines derived therefrom. As seen in Figs. 2B and 2C, the transformed cells all exhibited elevated EphA2 levels. Fig. 3 shows similar western blots, except using prostate cancer cell lines from dogs. As shown in Fig. 3, consistent with the results from human cells, EphA2 is overexpressed in metastatic prostatic carcinoma cells derived from dogs with spontaneous prostate cancer.

Please replace the paragraph beginning at page 11, line 5, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

It is believed that B2D6 decreases the growth of metastatic cells. Preliminary results reveal that B2D6 aggregates EphA2 and blocks about 50% of growth of metastatic breast cancer cells (which also overexpress EphA2) over the first four hours of incubation. Although EphA2 is not tyrosine phosphorylated in metastatic breast cancer cells, tyrosine phosphorylation is restored in these B2D6 treated cells. Thus, B2D6 is believed to restore normal EphA2 function.

Please replace the paragraph beginning at page 12, line 30, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

The present EphA2 antibodies, particularly those produced by hybridoma B2D6, are effective in blocking the growth and invasiveness of prostate cancer cells *in vivo*. The efficacy of B2D6 in blocking the growth of primary prostate tumors using subcutaneous implantation of PC3 tumor cells in mice is determined by use of subcutaneous models. The primary advantages of subcutaneous models are the ease of implantation and subsequent monitoring of tumor size. $5x10^5$ PC3 cells are inoculated subcutaneously into the right craniolateral thorax (axilla) using aseptic technique. Tumors are measured every 3-4 days using vernier calipers until they reach a volume of 0.2-0.3 cm^3. At that time, the mice are divided into four groups (8-10 animals each): Group 1 (vehicle control), Groups 2-4 are treated with 0.1, 1.0, or 10 mg/kg B2D6, administered intraperitoneally, twice a week. The mice are then monitored every 3 days to measure tumor volume (with vernier calipers), body weight, and life span. After no greater than 60 days past implantation, the animals are sacrificed and postmortem evaluations of tumorigenesis, including measurement and weight of implanted tumors and proximal lymph nodes, macroscopic evaluation of soft tissues for tumors (lymph nodes and lung), and formalin fixation of the primary tumor and tissues, are performed. The tissues are evaluated by immunohistochemistry using D7 (another EphA2 specific antibody that is amenable to immunohistochemistry) to determine the level of EphA2 expression in the tumors. In particular, tumor cells that escape B2D6 treatment are studied to determine whether they have low levels of EphA2 expression. Also, EphA2 expression in the individual animals is correlated with tumor invasiveness.

Please replace the paragraph beginning at page 18, line 7, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.